Arch Dermatol Res (2012) 304:619–625
DOI 10.1007/s00403-012-1237-1

ORIGINAL PAPER

Patented natural avocado sugar modulates the HBD-2 and HBD-3 expression in human keratinocytes through Toll-like receptor-2 and ERK/MAPK activation

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Received: 5 December 2011 / Revised: 19 March 2012 / Accepted: 31 March 2012 / Published online: 21 April 2012

Abstract Keratinocytes stimulated by microbial organisms secrete not only a variety of cytokines, chemokines and growth factors, but also antimicrobial peptides such as beta-defensins (HBDs) such as HBD-2 and HBD-3. AV119, a patented blend of avocado sugar, triggers the up-regulation of HBD-2 in skin epithelia upon contact with AV119, but the signalling mechanisms involved are not completely understood. The purpose of this study was to determine if AV119 was able to induce also the expression of HBD-3 in human keratinocytes. In addition, the receptor and intracellular pathways involved in the AV119 up-regulation of HBD-2 and HBD-3 were investigated. Our results demonstrated that AV119 induces a significantly increase of the expression of HBD-3. In addition, the HBD-2 and HBD-3 AV119-induced gene expression and release are TLR-2 dependent. Finally, we demonstrated that AV119 induced ERK/MAPK phosphorylation in human keratinocytes, thus providing evidence that HBD-2 and HBD-3 secretion is through the same transductional pathway. The ability of AV119 to induce also HBD-3 may amplify its therapeutic potential against a broader spectrum of bacterial and yeast strains responsible for human skin disorders.

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Keywords AV119 · β-defensins · Keratinocytes · MAPKs · TLRs

Abbreviations

HBDs	beta-defensins
MAPKs	Mitogen-activated protein (MAP) kinases
PAMPs	Pathogen-associated molecular patterns
TLRs	Toll-like receptors

Introduction

The epidermis plays an important role in the front line host defence against microbial infection. Keratinocytes, the major component of the epidermis, not only serve as a physical barrier, but also participate in the defence against pathogens. Keratinocytes stimulated by microbial organisms secrete not only a variety of cytokines, chemokines and growth factors, but also antimicrobial peptides such as beta-defensins (HBDs) [4]. HBDs are small cationic peptides which have antimicrobial activities and are secreted from epithelial cells, including keratinocytes [18]. HBD-2, first identified in psoriatic scales, is produced by epidermal keratinocytes and its production is enhanced in cutaneous infection, inflammatory dermatoses like psoriasis vulgaris, and wounds [6, 16]. HBD-2 kills Gram-negative bacteria and protects the skin from their infection [7]. Moreover, HBD-2 potentiates skin inflammation and wound repair; HBD-2 binds CC chemokine receptor-6, induces chemotaxis of memory T cells and immature dendritic cells and induces the production of IL-6, CC chemokine ligand-20 (CCL20), CXC chemokine ligand-10 and CCL5 in keratinocytes [16]. HBD-2 accelerates wound repair by inducing migration and proliferation of keratinocytes and endothelial cells [2, 16]. HBD-3 is also produced by epidermal

keratinocytes and, similarly to HBD-2, its release increases during inflammation [6, 16, 22]. HBD-3 has a salt-insensitive broad spectrum activity against Gram-positive and -negative bacteria or fungi like *Candida albicans,* thus protecting the skin from these types of infection [22]. It forms lytic pores on the bacterial lipid bilayers, especially *Staphylococcus aureus*, and kills them [13]. Moreover, HBD-3 accelerates skin inflammation by chemoattracting CC chemokine receptor-6 (CCR6)-positive cells [17] and inducing the production of interleukin-18 (IL-18), IL-8 and IL-20 in epidermal keratinocytes [10]. HBD-3 also promotes wound closure and reduces bacterial growth in *S. aureus*-infected diabetic wounds [20].

We previously demonstrated that contact of cultured epidermal keratinocytes with AV119, a patented blend of avocado sugars, triggers the up-regulation of HBD-2 [9]. However, the signalling mechanisms involved in the up-regulation of HBD-2 in skin epithelia upon contact with AV119 are not completely understood. The most widely studied innate recognition receptors are the Toll-like receptors (TLRs), a class of membrane receptors that sense microbes in the extracellular space or in intracellular compartments such as endosomes [1]. Although pathogen recognition begins at the level of the receptor, it is the signalling components downstream of each receptor and the ways in which they interact that ultimately determine the specific transcriptional response and immunological outcome [1]. Among the central pathways activated by immune innate signals are those involving the MAPKs. The MAPK family, which includes extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK) and p38 MAPK, is a family of serine/threonine kinases that play prominent roles in the immune system [8, 14]. The activation of MAPK is mediated by a core kinase module consisting of MAP kinase (MAPKKK, also known as MAP3K), MAP kinase (MAPKK, also known as MAP2K) and MAPK through sequential protein phosphorylation. Activated MAPKs, in turn, phosphorylate activating protein 1 (AP-1) transcription factors and other targets to stimulate gene transcription and immune responses [8, 14].

The purpose of this study was to determine if AV119 was able to induce also the expression of HBD-3 in human keratinocytes. In addition, the receptor and intracellular pathways involved in the AV119 up-regulation of HBD-2 and, possibly, HBD-3 were investigated.

Materials and methods

AV119 preparation

AV119 is a patented ingredient extracted from the *Avocado gratissima* fruit. After harvest, the avocado fruit is cut into slices and dried. The fats are eliminated and the dry matter is ground and extracted in a water-alcohol solution. Insoluble materials are then eliminated by filtration. The final product is obtained by concentrating the solution to obtain a 5 % content of active substance. This natural extract is mainly composed of two rare sugars, D-manno-heptulose and perseitol, which together represent 80 % of the dry matter.

Cell culture and treatments

Human keratinocytes were obtained from surgical specimens of normal adult skin and the concentration of total calcium in the medium was maintained at 0.09 mM. A transglutaminase assay was used to measure differentiation of the keratinocytes [9]. During the treatment, the concentration of total calcium in the keratinocyte serum-free medium (Keratinocyte-SFM; Life Technologies) was maintained at 0.12 mM. Semi-confluent keratinocytes (10^6/well) were treated or not treated with 25 μM SB203580 (p38 MAPK inhibitor), 25 μM SP600125 (JNK inhibitor), 10 μM U0126 (ERK inhibitor) for 1 h and with anti 10 μg/mL TLR-2 antibody (Santa Crutz) for 1 h and subsequently treated with 0.1 % AV119 for 24 and 48 h. The inhibitors were used at concentrations that resulted non-toxic to the cells. The synthetic diacylated lipoprotein (FSL-1), a ligand for TLR 2, was used as positive control to evaluate TLR2 induction.

Real-time PCR analysis for HBD-2 and HBD-3

Semi-confluent keratinocytes (10^6/well) were treated or not treated with SB203580 25 μM (inhibitor p38 MAPK), SP600125 25 μM (inhibitor JNK), U0126 10 μM (inhibitor ERK) for 1 h and with anti TLR-2 antibody 10 μg/mL (Santa Crutz) for 30 min and subsequently treated with 0.1 % AV119 for 24 and 48 h. At this time, total RNA was isolated with the High Pure RNA Isolation Kit (Roche Diagnostics). One hundred nanograms of total cellular RNA was reverse-transcribed (Expand Reverse Transcriptase, Roche Diagnostics) into complementary DNA (cDNA) using random hexamer primers (Random hexamers, Roche Diagnostics), at 42 °C for 45 min, according to the manufacturer's instructions. Real-time PCR was carried out with the LC Fast Start DNA Master SYBR Green kit (Roche Diagnostics) (LightCycler 2.0 Instrument) using 2 μl of cDNA, corresponding to 10 ng of total RNA in a 20-μl final volume, 3 mM $MgCl_2$ and 0.5 μM sense and antisense HBD-2 primers (5′-GGATCCATGG GTATAGGCGATCCTGTTA-3′; 5′-AAGCTTCTCTGAT GAGGGAGCCCTTTCT-3′) and HBD-3 primers (5′-AG CCTAGCAGCTATGAGGATC-3′; 5′-CTTCGGCAGCAT TTTGCGCCA-3′). The PCR condition for HBD-2 was

94 °C for 10′ (initial denaturation), followed by 40 cycles at 94 °C for 5″, 63 °C for 6″ and 72 °C for 10″. For HBD-3, it was 95 °C for 10′ followed by 45 cycles at 95 °C for 15″, 60 °C for 5″ and 72 °C for 10″. The annealing temperature was 63 °C. A melting curve was made at the end of each amplification to ensure the absence of non-specific reaction products. The accuracy of mRNA quantification depends on the linearity and efficiency of the PCR amplification. Both the parameters were assessed using standard curves generated by increasing amounts of cDNA. Quantification is based on the threshold cycle values, which are measured in the early stage of the exponential phase of the reaction, and by normalisation to the internal standard curve obtained with the housekeeping β-actin gene (5′-TGACGG GGTCACCCACACTGTGCCCATCTA-3′ and 5′-CTAGA AGCATTGCGGTGGACGATGGAGGG-3′, 60 °C), to avoid discrepancies in input RNA or in reverse transcription efficiency. The PCR products (respectively, 198 and 206 bp) were examined on 1.8 % agarose gel.

ELISA assay for HBD-2 and HBD-3

Semi-confluent keratinocytes (10^6/well) were treated or not treated with SB203580 25 μM (inhibitor p38 MAPK), SP600125 25 μM (inhibitor JNK), U0126 10 μM (inhibitor ERK) for 1 h and with anti TLR-2 antibody 10 μg/mL (Santa Crutz) for 1 h and subsequently treated with 0.1 % AV119 for 24 and 48 h. Cell-free supernatants were recovered by centrifugation and assayed for the HBD-2 and HBD-3 concentration by an enzyme-linked immunosorbent assay (Phoenix Pharmaceuticals, Inc.).

Protein extraction and western blotting analysis

Human keratinocytes were treated 0.1 % AV119 for 24 and 48 h. The cells were then scraped with 1 ml PBS and the cell pellet was homogenised with 300 ml of ice-cold buffer [137 mM NaCl, 1 % glycerol 10 %, 20 mM Tris–HCl pH 8, EDTA 5 mM, Triton X 100 1 %, 1 mM NaP_2O_7, 10 mM β-glycerophosphate $(CH_2OH)_2CHOP(O)(NaO)_2$] supplemented with 2 μg/ml of aprotinin, 20 μg/ml leupeptina, 1 mM phenylmethylsulfonyl fluoride (PMSF), 1 mM sodium orthovanadate, 10 mM NaF and 1 mM EGTA. The lysates were clarified by centrifugation at 10,000*g* for 30 min at 4 °C and assayed for protein content using Bradford's method [3]. Fifty micrograms of protein from each cell lysate was separated by 10 % SDS-PAGE under non-reducing conditions and transferred to nitrocellulose membranes. The filters were stained with a 10 % Ponceau S solution for 2 min to verify equal loading and transfer efficiency before being blocked with 5 % non-fat dry milk and incubated with the following antibodies: phospho-p38, phospho-JNK (Cell Signalling), phosho-p44/42 MAPK (ERK-1 and ERK-2), p44/42 MAPK (ERK-1 and ERK-2), p38 MAPK, JNK MAPK and TLR-2 (Santa Cruz) and β-tubulin (Sigma–Aldrich, St. Louis, MO, USA) overnight at 4 °C. After washing with 0.1 % Tween-20 PBS, the filters were incubated with an appropriate peroxidase-conjugated secondary antibody for 1 h at room temperature, washed thoroughly and analysed using the enhanced chemiluminescence detection system according to the manufacturer's recommendations (ECL Amersham, Amersham Pharmacia Biotech, UK).

Statistics

The results are expressed as the mean ± SD of the number of determinations indicated. Statistical analysis was performed using the XLSTAT 2009 for MS-Windows. The Student's *t* test was performed for each kind of experiment: the *p* value was generally evaluated to confirm the statistical significance of the results. A *p* value of 0.05 was considered to be significant.

Results

We previously demonstrated that AV119 induced the up-regulation of HBD-2 in human keratinocytes. The aim of the present study was to verify whether AV119 modulates the gene expression of another human beta-defensin, HBD-3. As shown in Fig. 1a and b, we demonstrated by real-time PCR and ELISA assay that AV119 induced a strong increase of both HBD-3 gene expression and protein levels after 48 h of treatment. To verify whether AV119 modulates the TLR gene expression, we first performed experiments to analyse which TLRs were expressed in primary human keratinocytes. Table 1 shows the results obtained by quantitative real-time PCR. Basal levels of mRNA were found for TLR-2, TLR-3, TLR-5 and TLR-9. No detectable signals were observed for TLR-1, TLR-4, TLR-6, TLR-7, TLR-8 or TLR-10. Human keratinocytes were subsequently treated with AV119 for 4 and 8 h. The results obtained by real-time PCR demonstrated that AV119 induced a strong increase of TLR-2 gene expression already after 4 h of treatment (Fig. 2a). The same result was obtained by western blot analysis (Fig. 2b). In contrast, no significant modulation was found for TLR3, TLR5 or TLR9 (data not shown). To determine whether the AV119-induced expression of HBD-2 and HBD-3 was TLR-2 dependent, human keratinocytes were pre-incubated with a TLR-2 neutralising antibody and then stimulated with AV119. As shown in Figs. 1a and 3a, the TLR-2 neutralizing antibody significantly inhibited the AV119-induced HBD-2 and HBD-3 expression, respectively, in human keratinocytes. The antibody alone did not modulate

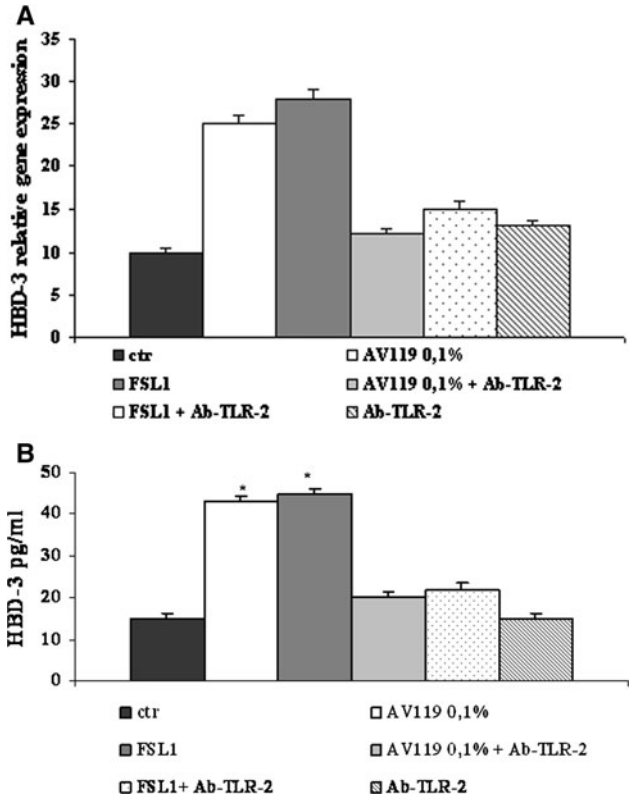

Fig. 1 **a** HBD-3 mRNA expression in human keratinocytes pre-treated with anti TLR-2 antibody 10 μg/mL for 1 h followed by treatment with AV119. The mRNA expression was analysed by real-time PCR. All quantifications were normalised to the housekeeping gene β-actin. The data are presented as the ratio between the target gene expression and the gene expression in unstimulated conditions. **b** HBD-3 protein expression in human keratinocytes pre-treated with anti TLR-2 antibody 10 μg/mL for 1 h followed by treatment with AV119. The data shown are representative of three different experiments ± SD ($*p < 0.05$)

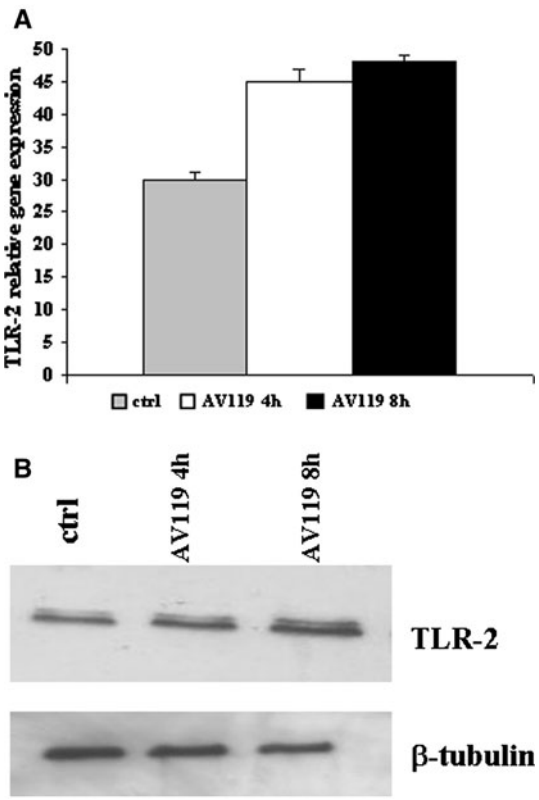

Fig. 2 **a** AV119 induced TLR-2 expression in human keratinocytes: TLR-2 mRNA expression analysed by real-time PCR in human keratinocytes treated with AV119. All quantifications were normalised to the housekeeping gene β-actin. The data are presented as the ratio between the target gene expression and the gene expression in unstimulated conditions. **b** TLR-2 protein expression in human keratinocytes treated with AV119. β-tubulin was used as internal control of protein load. The picture is the representative of three different experiments

Table 1 TLRs gene expression analysed by real-time PCR in human keratinocytes

TLRs										
RNA source	1	2	3	4	5	6	7	8	9	10
Human keratinocytes	−	+	+	−	+	−	−	−	+	−

The Toll receptors expressed in keratinocytes are indicated by the symbol +, − no detectable gene expression

the HBD-2 and -3 gene expression in treated keratinocytes. ELISA assay confirmed the results obtained by real-time PCR (Figs. 1b, 3b, respectively). To identify the TLR-2-mediated downstream signalling pathways involved in HBD-2 and HBD-3 induction, p38 MAP kinase inhibitor SB203580, c-Jun NH2-terminal kinase [JNK] inhibitor SP600125 and ERK kinase inhibitor U0126 were used. In brief, human keratinocytes were pre-treated for 1 h with kinase inhibitors and subsequently stimulated with AV119. Real-time PCR results indicated that only in the presence of ERK kinase inhibitor did HBD-2 and HBD-3 mRNA levels return to being comparable to the control (Figs. 4a, 5a). The kinase inhibitors alone did not modulate the HBD-2 and -3 gene expression in treated keratinocytes. ELISA assay was performed to confirm the results obtained by real-time PCR. Figures 4b and 5b show the reduced accumulation of HBD-2 and HBD-3, respectively, in the supernatants of keratinocytes pre-treated with ERK inhibitor and subsequently treated with AV119. Western blot confirmed that ERK is the kinase involved in AV119-induced HBD-2 and HBD-3 expression (Fig. 6).

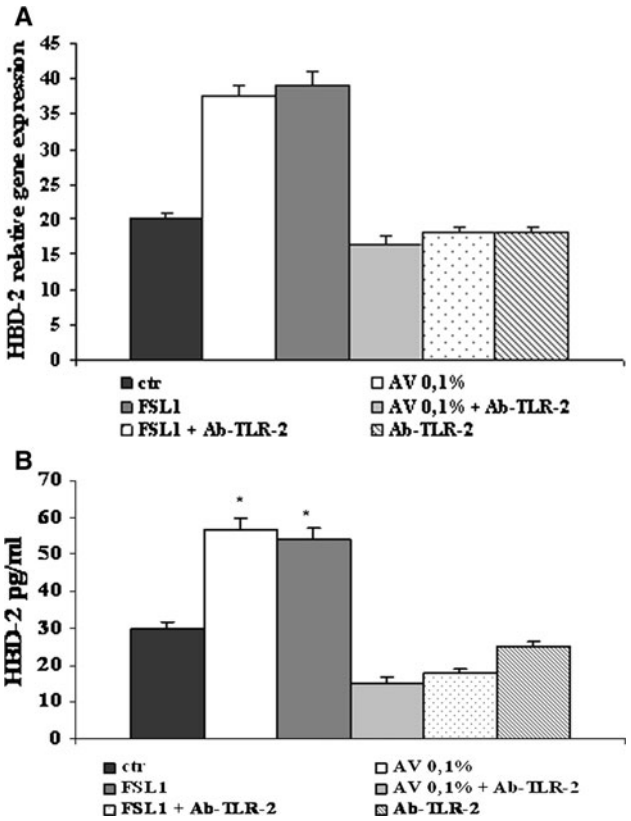

Fig. 3 **a** HBD-2 mRNA expression in human keratinocytes pre-treated with anti TLR-2 antibody 10 μg/mL for 1 h followed by treatment with AV119. The mRNA expression was analysed by real-time PCR. All quantifications were normalised to the housekeeping gene β-actin. The data are presented as the ratio between the target gene expression and the gene expression in unstimulated conditions. **b** HBD-2 protein expression in human keratinocytes pre-treated with anti TLR-2 antibody 10 μg/mL for 1 h followed by treatment with AV119. The data shown are representative of three different experiments ± SD ($*p < 0.05$)

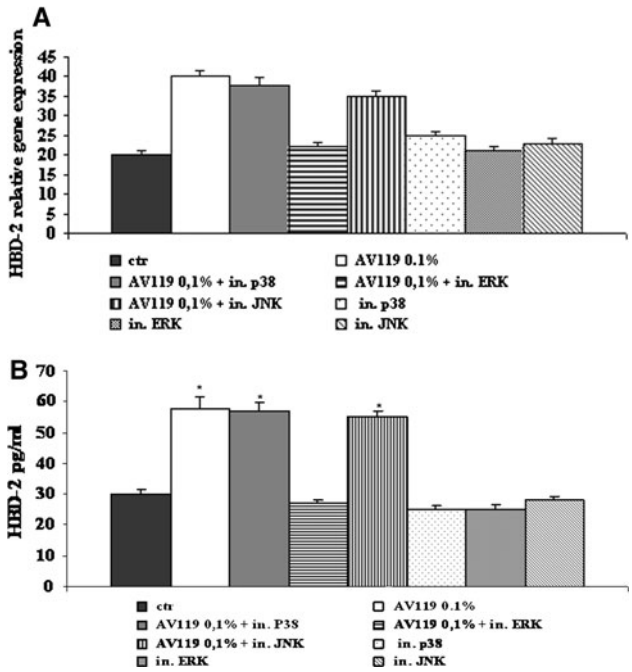

Fig. 4 **a** HBD-2 mRNA expression in human keratinocytes were pre-treated for 1 h with kinase inhibitors and subsequently stimulated with AV119. The mRNA expression was analysed by real-time PCR. All quantifications were normalised to the housekeeping gene β-actin. The data are presented as the ratio between the target gene expression and the gene expression in unstimulated conditions. **b** HBD-2 protein expression in human keratinocytes were pre-treated for 1 h with kinase inhibitors and subsequently stimulated with AV119. The data shown are representative of three different experiments ± SD ($*p < 0.05$)

Discussion

Resistance to infection of the skin has been shown in the recent years to be based on the function of intact innate immune mechanisms by epidermal keratinocytes such as the production of antimicrobial pore-forming peptides of the human β-defensin family. β-defensins are up-regulated by a multitude of stimuli through signal transduction pathways utilising nuclear transcription factors (NF)-κB or AP-1 and the MAPK system [17].

We recently demonstrated that the contact of cultured epidermal keratinocytes with AV119 triggers the up-regulation of HBD-2 [9]. In addition, we showed that AV119 induced the nuclear translocation of AP-1 and that both the *fos* and *jun* family proteins were involved, thus supporting a role for AP-1 in mediating HBD-2 gene transcription [19].

Here, we showed that AV119 was able to modulate also the HBD-3 gene expression. This result is of interest since HBD-3 has a broader activity spectrum against Gram-positive and -negative bacteria. In particular, HBD-3 protects the skin from colonisation of *S. aureus*, the leading cause of skin and soft tissue infections such as abscesses, furuncles and cellulites [21].

Receptors of the TLR family are important factors of host innate immune cells that help to promote an adaptive immune response. They are expressed on cells of the epithelial lineage such as intestinal epithelial cells or human keratinocytes [11]. In humans, ten TLR family members have been identified [12]. Our results demonstrated that basal levels of TLR-2, TLR-3, TLR-5 and TLR-9 were expressed in primary human keratinocytes. Of these TLRs, AV119 had the ability to modulate only TLR-2 gene expression. In addition, the HBD-2 and HBD-3 AV119-induced gene expression and release were abrogated using a TLR-2 neutralizing antibody.

Mediators of downstream signalling pathways of TLRs include MAPKs. The MAPKs are related enzymes that

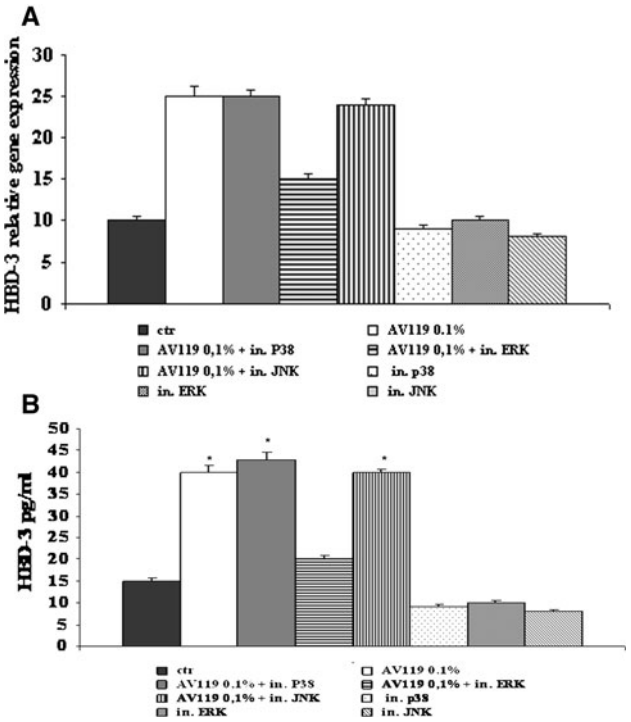

Fig. 5 **a** HBD-3 mRNA expression in human keratinocytes were pre-treated for 1 h with kinase inhibitors and subsequently stimulated with AV119. The mRNA expression was analysed by real-time PCR. All quantifications were normalised to the housekeeping gene β-actin. The data are presented as the ratio between the target gene expression and the gene expression in unstimulated conditions. **b** HBD-3 protein expression in human keratinocytes were pre-treated for 1 h with kinase inhibitors and subsequently stimulated with AV119. The data shown are representative of three different experiments ± SD ($*p < 0.05$)

respond to extracellular stimulation through direct MAPK-dependent activation, loop phosphorylation and kinase activation [5]. Here, we demonstrate that AV119 induced ERK/MAPK phosphorylation in human keratinocytes, thus providing evidence that HBD-2 and HBD-3 secretion is through the same transductional pathway. In fact, U0126, a well-characterised chemical inhibitor of ERK but not SB203580 or SP600125; p38 and JNK inhibitors, almost completely nullified both HBD-2 and HBD-3 mRNA expression and protein secretion, respectively.

Sugars in the form of monosaccharides, oligosaccharides, polysaccharides and glycoconjugates (glycoproteins and glycolipids) are vital components of infecting microbes and host cells, and play important roles in microbial adherence and colonisation, biofilm formation and virulence. TLRs enable the host to recognise pathogen-associated molecular patterns (PAMPs), which are mainly glycolipids. It is known that the interaction of TLRs with PAMPs initiates a cascade of events that leads to the production of reactive oxygen intermediates, cytokines and chemokines and promotes inflammation and immunomodulation. Lloyd et al. [15] suggested that exogenous sugars can block carbohydrate receptors and competitively displace bacteria from attachment to cells, including keratinocytes, and, therefore, sugars may provide valuable adjunctive anti-inflammatory and/or antimicrobial treatment. They conclude affirming that carbohydrate molecules might be used to stimulate the production of antimicrobial substances and/or to exert specific interference with TLRs. In this context, AV119 might work competitively to displace pathogenic micro-organisms from attachment to keratinocytes and, at the same time, promote the antimicrobial defence of the host through a TLR-2 dependent HBD-2 and HBD-3 production, but in the absence of proinflammatory response, as previously reported [9].

In conclusion, the present study demonstrated that AV119 induces the expression of HBD-3, as well as HBD-2, through TLR-2 activation and ERK/MAPK pathway involvement. In addition, the ability of AV119 to induce also HBD-3 may amplify its therapeutic potential against a broader spectrum of bacterial and yeast strains responsible for human skin disorders.

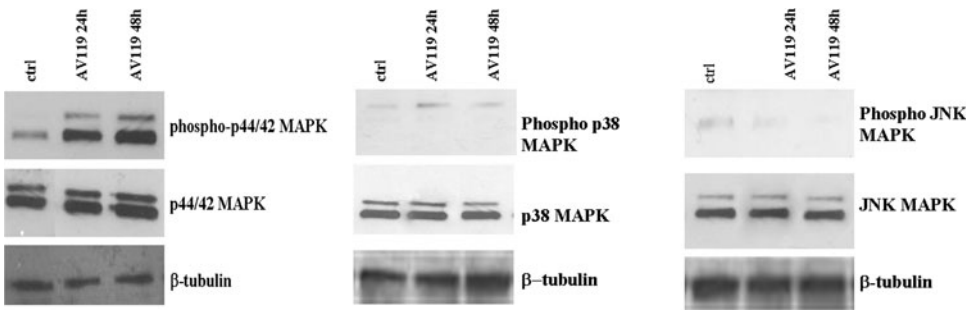

Fig. 6 AV119-induced the ERK/MAPK phosphorylation: Human keratinocytes were treated or not treated with AV119. Protein extracts were analysed using antibodies to p44/42 MAPK (ERK-1 and ERK-2), p38 MAPK, JNK MAPK and phospho-p44/42 MAPK (ERK-1 and ERK-2) phospho-p38 MAPK, phosphor-c-Jun MAPK as described in "Methods". β-tubulin was used as internal control of protein load. The picture is the representative of three different experiments

Acknowledgments This study was supported by grants from Expanscience Laboratoires, R&D Centre, Epernon, France.

References

1. Akira S, Uematsu S, Takeuchi O (2006) Pathogen recognition and innate immunity. Cell 124:783–801
2. Baroni A, Donnarumma G, Paoletti I, Longanesi-Cattani I, Bifulco K, Tufano MA, Carriero MV (2009) Antimicrobial human beta-defensin-2 stimulates migration, proliferation and tube formation of human umbilical vein endothelial cells. Peptides 30(2):267–272
3. Bradford MA (1976) Rapid and sensitive method for the quantification of microgram quantities of protein utilising the principle of protein binding. Anal Bioch 72:248–256
4. Braff MH, Bardan A, Nizet V, Gallo RL (2005) Cutaneous defense mechanisms by antimicrobial peptides. J Investig Dermatol 125:9–13
5. Cargnello M, Roux PP (2011) Activation and function of the MAPKs and their substrates, the MAPK-activated protein kinases. Microbiol Mol Biol Rev 75(1):50–83
6. De Jongh GJ, Zeeuwen PL, Kucharekova M, Pfundt R, van der Valk PG, Blokx W, Dogan A, Hiemstra PS, van de Kerkhof PC, Schalkwijk J (2005) High expression levels of keratinocyte antimicrobial proteins in psoriasis compared with atopic dermatitis. J Invest Dermatol 125:1163–1173
7. Dinulos JG, Mentele L, Fredericks LP, Dale BA, Darmstadt GL (2003) Keratinocyte expression of human beta defensin 2 following bacterial infection: role in cutaneous host defense. Clin Diagn Lab Immunol 10:161–166
8. Dong C, Davis RJ, Flavell RA (2002) MAP kinases in immune response. Ann Rev Immunol 20:55–72
9. Donnarumma G, Buommino E, Baroni A, Auricchio L, De Filippis A, Cozza V, Msika P, Piccardi N, Tufano MA (2007) Effects of AV119, a natural sugar from avocado, on *Malassezia furfur* invasiveness and on the expression of HBD-2 and cytokines in human keratinocytes. Exp Dermatol 16(11):912–919
10. Hirsch T, Spielmann M, Zuhaili B, Fossum M, Metzig M, Koehler T, Steinau HU, Yao F, Onderdonk AB, Steinstraesser L, Eriksson E (2009) Human β-defensin-3 promotes wound healing in infected diabetic wounds. J Gene Med 11:220–228
11. Kawai K, Shimura H, Minagawa M, Ito A, Tomiyama K, Ito M (2002) Expression of functional Toll-like receptor 2 on human epidermal keratinocytes. J Dermatol Sci 30:185–194
12. Kawai T, Akira S (2010) The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. Nat Immunol 11:373–384
13. Kisich KO, Howell MD, Boguniewicz M, Heizer HR, Watson NU, Leung DY (2007) The constitutive capacity of human keratinocytes to kill *Staphylococcus aureus* is dependent on β-defensin 3. J Invest Dermatol 127:2368–2380
14. Kyriakis JM, Avruch J (2001) Mammalian mitogen-activated protein kinase signal transduction pathways activated by stress and inflammation. Physiol Rev 81:807–869
15. Lloyd DH, Viac J, Werling D, Rème CA, Gatto H (2007) Role of sugars in surface microbe–host interactions and immune reaction modulation. Vet Dermatol 18(4):197–204 Review
16. Niyonsaba F, Ushio H, Nakano N, Ng W, Sayama K, Hashimoto K, Nagaoka I, Okumura K, Ogawa H (2007) Antimicrobial peptides human-defensins stimulate epidermal keratinocyte migration, proliferation and production of proinflammatory cytokines and chemokines. J Invest Dermatol 127:594–604
17. Niyonsaba F, Ushio H, Nagaoka I, Okumura K, Ogawa H (2005) The human beta-defensins (-1, -2, -3, -4) and cathelicidin LL-37 induce IL-18 secretion through p38 and ERK MAPK activation in primary human keratinocytes. J Immunol 175:1776–1784
18. Nizet V, Ohtake T, Lauth X, Trowbridge J, Rudisill J, Dorschner RA, Pestonjamasp V, Piraino J, Huttner K, Gallo RL (2001) Innate antimicrobial peptide protects the skin from invasive bacterial infection. Nature 414:454–457
19. Paoletti I, Buommino E, Tudisco L, Baudouin C, Msika P, Tufano MA, Baroni A, Donnarumma G (2010) Patented natural avocado sugars modulate the HBD-2 expression in human keratinocytes through the involvement of protein kinase C and protein tyrosine kinases. Arch Dermatol Res 302(3):201–209
20. Röhrl J, Yang D, Oppenheim JJ, Hehlgans T (2008) Identification and biological characterization of mouse β-defensin 14, the orthologue of human beta-defensin 3. J Biol Chem 283(9): 5414–5419
21. Sass V, Schneider T, Wilmes M, Körner C, Tossi A, Novikova N, Shamova O, Sahl HG (2010) Human beta-defensin 3 inhibits cell wall biosynthesis in Staphylococci. Infect Immun 78(6): 2793–2800
22. Sawamura D, Goto M, Shibaki A, Akiyama M, McMillan JR, Abiko Y, Shimizu H (2005) β defensin-3 engineered epidermis shows highly protective effect for bacterial infection. Gene Ther 2:857–861